

tive feature not reported previously in any species of *Accacladocoelium*. This feature raises the possibility of an undescribed species within the genus.

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Literature Cited

- Alvarino, A. 1964. Bathymetric distribution of chaetognaths. *Pacific Science* 18:64–82.
- . 1965. Chaetognaths. in Harold Barnes, ed. *Annual Review of Oceanography and Marine Biology*. 3:115–195.
- Dawes, B. 1958. *Sagitta* as a host of larval trematodes, including a new and unique type of cercaria. *Nature* 182:960–961.
- . 1959. On *Cercaria owreae* (Hutton, 1954) from *Sagitta hexaptera* (d'Orbigny) in the Caribbean plankton. *Journal of Helminthology* 33:209–222.
- Furnest, M. L., and J. Rebecq. 1966. Sur l'ubiquité de *Cercaria owreae* (R. F. Hutton, 1954). *Annales de Parasitologie* 41:61–70.
- Hutton, R. F. 1952. Schistosome cercariae as the probable cause of seabather's eruption. *Bulletin of Marine Science of the Gulf and Caribbean* 2:346–359.
- . 1954. *Metacercaria owreae* n. sp. an unusual trematode larvae from the Florida Current. *Chaetognaths*. *Bulletin of Marine Science of the Gulf and Caribbean* 4:104–109.
- Ödhner, T. 1911. Zum natürlichen System der digenen Trematoden. *Zoologischer Anzeiger* 4:513–531.
- Margolis, L., G. W. Esch, J. C. Holmes, A. M. Kuris, and G. A. Schad. 1982. The use of ecological terms in parasitology. *Journal of Parasitology* 68:131–133.
- Suárez-Caabro, J. A. 1955. Quetognatos de los mares Cubanos. *Memorias de la Sociedad Cubana de Historia Natural* 22:125–180.

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Research Note

New Host and Locality Records for Three Species of *Glypthelmins* (Digenea: Macroderoididae) in Anurans of Mexico

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ABSTRACT: During an inventory of the helminth parasites of amphibians from several localities in Mexico, trematode parasites of the genus *Glypthelmins* from 5 species of frogs were studied. Three species of *Glypthelmins* were collected from *Rana montezumae*, *Rana dunni*, *Rana neovolcanica*, *Rana megapoda*, and *Rana vaillanti*. New host and locality records for *Glypthelmins quieta* and *Glypthelmins californiensis* in anurans from Mexico are established, and we report *Glypthelmins facioi* for the first time from *R. vaillanti* from Los Tuxtlas, Veracruz State. Diagnostic characters for each parasite species and sister-group relationships are presented.

KEY WORDS: Digenea, Macroderoididae, *Glypthelmins* spp., anurans, systematics, frogs, *Rana* spp., Mexico.

The genus *Glypthelmins* was established by Stafford (1905) to include *Distomum quietum* Stafford, 1900, parasitic in *Rana catesbeiana* Shaw, 1802, *Rana virescens* Kalm, 1878, and *Hyla pickeringii* Holb, 1890, all from Canada. At the present time there is controversy about the species comprising the genus *Glypthelmins*, primarily because the original description of the type species of *Glypthelmins* was incomplete. This, and some degree of intraspecific morphological variability among some members of the genus, have led to taxonomic uncertainty concerning the species. This confusion has resulted in investigators creating nonphylogenetic groups, and some species that should be included in *Glypthelmins* were assigned to other gen-

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Table 1. Prevalence and mean abundance of 3 species of *Glythelmins* in 5 species of frogs from Mexico.

Host	Locality*	N†	<i>Glythelmins</i>		
			<i>californiensis</i>	<i>quieta</i>	<i>facioi</i>
<i>Rana montezumae</i>	CLE	89	330‡/23.6§ (3.7)	1034/39.3 (11.6)	—
<i>Rana dunni</i>	LZA	73	273/46.6 (3.7)	39/15 (3.5)	—
	LPA	18	4/22.2 (1)	180/50 (20)	—
<i>Rana megapoda</i>	LCU	46	—	6/8.7 (0.13)	—
<i>Rana neovolcanica</i>	MCO	34	—	231/31.7 (5.6)	—
<i>Rana vaillanti</i>	LAE	34	—	—	31/41.2 (0.91)

* CLE = Ciénaga de Lerma; LZA = Lago de Zacapu; LPA = Lago de Pátzcuaro; LCU = Lago de Cuitzeo; MCO = Manantiales de Cointzio; LAE = Laguna Escondida.

† N = sample size.

‡ Number of worms collected.

§ Prevalence of infection (expressed as %).

|| Mean abundance of infection (mean no. of worms per host examined).

era such as *Margeana* Cort, 1919, *Microderma* Mehra, 1931, *Choledocystus* Pereira and Cuocolo, 1941, *Rauschiella* Babero, 1951, *Reynoldstrema* Cheng, 1959, and *Repandum* Byrd and Maples, 1963 (Miller, 1930; Caballero, 1938; Cheng, 1959; Byrd and Maples, 1963). In Mexico, at least 4 species of the genus have been reported from frogs and toads: *Glythelmins californiensis* (Cort, 1919) Miller, 1930, *Glythelmins quieta* (Stafford, 1900) Stafford, 1905, *Glythelmins intermedia* (Caballero, Bravo, and Zerecero, 1944) Yamaguti, 1958 (= *Choledocystus intermedia*), and *Glythelmins tineri* (Babero, 1951) Brooks, 1977 (= *Rauschiella tineri*) (see Lamothe-Argumedo et al., 1997; Brooks, 1977). As part of an ongoing inventory of the helminth parasites of amphibians from different localities in Mexico, we establish herein new host and locality records for 3 species of *Glythelmins*. During this study, we examined the species of *Glythelmins* deposited at the Colección Nacional de Helmintos (CNHE) and produced a revised list of hosts and species in Mexico.

Between 1996 and 1997, individuals of 18 species of frogs and toads were collected from 9 localities in Mexico. Only at 5 of these localities (Ciénaga de Lerma, Estado de México [CLE], 19°17'N, 99°30'W; Lago de Pátzcuaro, Michoacán [LPA], 19°30'N, 101°36'W; Lago de Zacapu, Michoacán [LZA], 19°49'N, 101°47'W; Manantiales de Cointzio, Michoacán [MCO], 19°35'N, 101°14'W; and Laguna Escondida, Los Tuxtlas, Veracruz [LAE], 20°37'N, 98°12'W), and only in 5 of the 18 species of frogs and toads studied were several specimens of *Glypt-*

helmins recovered from the intestines of their hosts. Anurans were captured by hand, and in <12 hr they were killed with an overdose of sodium pentobarbital. All organs and the body cavities of each host were examined for helminths using a stereomicroscope. Digeneans recovered from the intestine were initially placed in 7.5% saline and were subsequently fixed in Bouin's fluid for 12 hr under a coverglass. Some worms were mounted as semipermanent slides in saline and studied alive. Morphological analyses were conducted using an image analyzer (Image-Pro Plus version 1.3 for Windows). Voucher specimens have been deposited at the Colección Nacional de Helmintos, Mexico City (accession numbers 3271–3285), and at the Harold W. Manter Laboratory of Parasitology (HWMML), University of Nebraska–Lincoln, Nebraska, U.S.A. (accession numbers 39954–39957).

Three species of *Glythelmins* were found: *G. californiensis*, 607 specimens from 2 species of frogs (*Rana montezumae* Baird, 1854, CLE; *Rana dunni* Zweifel, 1957, LPA and LZA); 1,484 specimens of *G. quieta* from 4 species of frogs (*R. montezumae*, CLE; *R. dunni*, LPA and LZA; *Rana neovolcanica* Hillis and Frost, 1985, MCO; *Rana megapoda* Taylor, 1942, MCO); and 31 specimens of *Glythelmins facioi* from *Rana vaillanti* Brocchi, 1877, in LAE. Infection data are in Table 1.

The 3 species found show morphological traits that are typical of *Glythelmins*, including the presence of 2 symmetric or oblique intercecal testes, a median genital pore, the presence of a seminal receptacle, bipartite seminal vesicle,

and an I- or Y-shaped excretory vesicle. *Glypthelmins quieta* is characterized by having 2 groups of prominent peripharyngeal glands on each side of the pharynx extending to the cecal bifurcation, with gland ducts opening at the posterior border of the oral sucker. Vitelline follicles extend from the posterior border of the pharynx and occasionally from the midlevel of the esophagus, reaching far beyond the posterior border of the testes. In addition, *G. quieta* possesses cecal, intracecal, and extracecal uterine loops.

The original description of *G. californiensis* by Cort (1919), based on live specimens, indicated the absence of peripharyngeal glands. We have studied specimens identified as *G. californiensis* from CNHE (nos. 3280–3284) and from the personal collection of Dr. Daniel Brooks from *Rana aurora* Baird and Girard, 1852, from British Columbia, Canada. These specimens possess reduced peripharyngeal glands that surround the pharynx both ventrally and dorsally. Because the location of the holotype of this species is not known, we are unable to confirm this characteristic until a neotype is assigned and studied. However, our observations agree with those made by O'Grady (1987) who described *G. californiensis* from British Columbia, naming these glands as medial glands. *Glypthelmins californiensis* has vitelline follicles that extend anteriorly to the level of the posterior border of the pharynx and occasionally to the posterior border of the oral sucker with follicles confluent dorsally at the cecal bifurcation. The vitellaria extend to the posterior border of the testes. Uterine loops are completely intracecal. In contrast, *G. facioi* is characterized by lacking peripharyngeal glands, vitelline follicles extending anteriorly from the cecal bifurcation just beyond the posterior border of the left testis, by having oblique rather than symmetric testes, cecal and intracecal uterine loops, and by having tegumentary spines that extend only along the anterior $\frac{2}{3}$ of the body.

These 3 species of *Glypthelmins* constitute a monophyletic clade, according to the phylogenetic hypothesis proposed by Brooks (1977) and Brooks and McLennan (1993). *Glypthelmins facioi* is the sister species of the species pair *G. quieta* + *G. californiensis*. *Glypthelmins facioi* was originally described from *R. pipiens* Schreber, 1782, from Costa Rica by Brenes et al. (1959), and later redescribed by Sullivan (1976). Herein, we report *G. facioi* for the first time

from Mexico, thus establishing a new host and locality record. Based on previous geographical records, this species is apparently restricted to the neotropics. *Glypthelmins quieta*, the type species of the genus, is widely distributed in North America, including the eastern U.S.A., Canada, and Central Mexico, parasitizing at least 21 species of anurans in 5 genera (*Acris* Dumeril and Bibron, 1841, *Bufo* Laurenti, 1768, *Hyla* Laurenti, 1768, *Pseudacris* Fitzinger, 1843, and *Rana* Linnaeus, 1758). In Mexico, this species was previously recorded from *R. montezumae* from Xochimilco and Texcoco lakes, both in the vicinity of Mexico City (Lamothe-Argumedo et al., 1997). In this report we add 4 new locality records (CLE, LPA, LZA, MCO), and 3 host records, all belonging to the *R. pipiens* complex (leopard frogs) including *R. dunni*, *R. neovolcanica*, and *R. megapoda*.

Glypthelmins californiensis also occurs in the Nearctic Region but has a different geographic distribution than the type species; it occurs in North America, but is known only from 6 species of *Rana* and 1 species of *Hyla*. Its range extends through the western U.S.A. and Canada, converging with *G. quieta* in frogs from the central region of Mexico in localities of the Transverse Neovolcanic Axis, at the boundary between the Nearctic and Neotropical biogeographic zones. Previously, this species was reported in Mexico from *R. montezumae* and *R. pipiens* from Mexico City and Lerma (Caballero, 1942; Caballero and Sokoloff, 1934; León-Régagnon, 1992) and from *R. dunni* from Lake Patzcuaro (Pulido, 1994). Herein, we establish Lake Zacapu as a new locality record for *G. californiensis*. Guillén (1992) recorded *G. californiensis* as a parasite of *Rana berlandieri* Baird, 1854, and *R. vaillanti* from Los Tuxtlas, Veracruz State. We examined specimens deposited at the CNHE (no. 1514, 5 specimens). Based on our diagnoses of the 3 species, we believe these were misidentified because in them the vitellaria extend anteriorly to the level of cecal bifurcation, and posteriorly they extend to the posterior border of the testes. The specimens do have oblique testes, and the uterine loops are intra- and extracecal. In our opinion, they are *G. facioi*.

As can be generally expected, the close phylogenetic relationship between *G. quieta* and *G. californiensis* (see Brooks, 1977; Brooks and McLennan, 1993) determines some degree of

Table 2. Species of *Glypthelmins* recorded from anurans from Mexico.

Species	Host	Locality	Reference
<i>Glypthelmins californiensis</i> *	<i>Rana montezumae</i> ,	México, Distrito Federal	Caballero and Sokoloff (1934)
	<i>Rana pipiens</i>	Xochimilco, Distrito Federal	
	<i>R. montezumae</i> , <i>R. pipiens</i>	Ciénaga de Lerma, Estado de México	Caballero (1942)
	<i>Rana dunni</i>	Lago de Pátzcuaro, Michoacán	
<i>Glypthelmins facioi</i> *	<i>Rana vaillanti</i> , <i>Rana berlandieri</i>	Lago de Zacapu, Michoacán	Pulido (1994)
		Laguna Escondida, "Los Tuxtlas", Veracruz	This work
<i>Glypthelmins intermedia</i> †‡§	<i>Bufo marinus</i>	Rio Huixtla, Chiapas	This work; Guillen (1992)
		Tuxtepec, Oaxaca	Caballero et al. (1944)
<i>Glypthelmins quieta</i> *	<i>R. dunni</i>	Lago de Pátzcuaro and Lago de Zacapu, Michoacán	Bravo (1948)
		Lago de Cuitzeo, Michoacán	This work
	<i>Rana megapoda</i>	Manantiales de Cointzio, Michoacán	This work
	<i>Rana neovolcanica</i>	Ciénaga de Lerma, Estado de México	This work
	<i>R. montezumae</i>	San Pedro Tlaltizapan, Estado de México	León-Règagnon (1992)
<i>Glypthelmins tineri</i> *	"Green frog"	Xochimilco, Distrito Federal and Texcoco, Estado de México	Lamothe-Argumedo et al. (1997)
		Mexico	Babero (1951)

* Intestine.

† Liver.

‡ Gall bladder.

§ Bile ducts.

|| Locality not determined.

morphological similarity. Detailed examination of diagnostic characters allowed us to review the taxonomic status of species of *Glypthelmins* deposited at the CNHE. We examined specimens from the following lots: lot no. 1561 representing 10 specimens from *R. dunni* from Lake Patzcuaro, identified by Pulido (1994) and labeled as *G. californiensis* (1 individual is actually *G. quieta*); lot no. 1461, represented by 8 specimens from *R. montezumae* identified by León-Règagnon (1992) from Lerma, and labeled as *G. californiensis*, are *G. quieta*; lot no. 1181, 17 specimens from *R. montezumae* from Lerma, collected and identified by Caballero (1942); and lot no. 2495, represented by 8 specimens from *R. montezumae* from Lake Xochimilco, identified by Dr. Eduardo Caballero, were correctly identified as *G. californiensis*; lots no. 1562 (3 specimens) and 1563 (4 specimens), from *R. montezumae* from Lake Xochimilco and Lake Texcoco, respectively, were correctly identified as *G. quieta*.

In Table 2, we present an updated and revised list of species of *Glypthelmins* in anurans from Mexico. Adding previous records to the results, we conclude the genus *Glypthelmins* is currently represented in Mexico by 5 species (*G. quieta*,

G. californiensis, *G. facioi*, *G. intermedia*, and *G. tineri*) from at least 7 species of *Rana* and 1 species of *Bufo*. The most common of these are *G. californiensis* and *G. quieta*, both found in different species of frogs in localities of the Mesa Central of Mexico. Whether or not these are all the species of *Glypthelmins* that occur in anurans from Mexico will be determined once further research on the helminth fauna of different species of amphibians in the country is finished.

The species composition of the genus *Glypthelmins*, as well as its taxonomic position and relationships to other closely related genera, are still uncertain. Yamaguti (1971) recognized 23 valid species; Brooks (1977) in his phylogenetic analysis of species of *Glypthelmins*, considered 19 species to be valid. Prudhoe and Bray (1982) proposed that some species, allocated originally to other genera, should be transferred to *Glypthelmins*, and then included 27 species in the genus. A complete revision of the genus is necessary to clarify the taxonomic composition of this group of parasites as well as to update the phylogenetic hypotheses of Brooks (1977) and Brooks and McLennan (1993). We are currently obtaining DNA sequences of 18S ribosomal

genes as an additional source of characters. Preliminary results show an agreement of sister-group relationships among the 3 species discussed here.

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Literature Cited

- Babero, B. B.** 1951. *Rauschiella tineri* n. g., n. sp. a trematode (Plagiorchiinae) from a frog. *Journal of Parasitology* 37:560–562.
- Bravo, H. M.** 1948. Descripción de dos especies de tremátodos parásitos de *Bufo marinus* L. procedentes de Tuxtepec, Oaxaca. *Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoológica* 19:153–161.
- Brenes, M. R., G. S. Arroyo, O. Jiménez-Quiroz, and E. Delgado-Flores.** 1959. Algunos tremátodos de *Rana pipiens*. Descripción de *Glypthelmins facioi* n. sp. *Revista de Biología Tropical* 72:191–197.
- Brooks, R. D.** 1977. Evolutionary history of some plagiorchioid trematodes of anurans. *Systematic Zoology* 26:277–289.
- , and **D. McLennan.** 1993. *Parascript: Parasites and the Language of Evolution*. Smithsonian Institution Press, Washington, D.C. 429 pp.
- Byrd, E. E., and W. P. Maples.** 1963. The glypthelminths (Trematoda: Digenea), with a redescription of one species and the erection of a new genus. *Zeitschrift für Parasitenkunde* 22:521–536.
- Caballero, C. E.** 1938. Contribución al conocimiento de la helmintofauna de México. Tesis Doctoral, Facultad de Filosofía y Estudios Superiores, Universidad Nacional Autónoma de México. 149 pp.
- , 1942. Tremátodos de las ranas de la Ciénaga de Lerma, Estado de México. III. Redescrípción de una forma norteamericana de *Haematoloechus* y algunas consideraciones sobre *Glypthelmins californiensis* (Cort, 1919). *Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoológica* 13:71–79.
- , **M. H. Bravo, and C. Zerecero.** 1944. Estudios helmintológicos de la región oncocercosa de México y de la República de Guatemala. Trematoda I. *Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoológica* 15:59–72.
- , and **D. Sokoloff.** 1934. Tercera contribución al conocimiento de la parasitología de *Rana montezumae*. *Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoológica* 5:337–340.
- Cheng, T. C.** 1959. Studies on the trematode family Brachycoeliidae, II. Revision of the genera *Glypthelmins* (Stafford, 1900) Stafford, 1905, and *Margreana* Cort, 1919; and the description of *Reynoldstrema* n. g. (Glypthelminae, n. subfam.). *American Midland Naturalist* 61:68–88.
- Cort, W. W.** 1919. A new distome from *Rana aurora*. *University of California Publications in Zoology* 8:283–298.
- Guillén, H. S.** 1992. Comunidades de helmintos de algunos anuros de “Los Tuxtlas”, Veracruz. Master's Thesis, Facultad de Ciencias, Universidad Nacional Autónoma de México. 90 pp.
- Lamothe-Argumedo, R., L. García-Prieto, D. Osorio-Sarabia, and G. Pérez-Ponce de León.** 1997. Catálogo de la Colección Nacional de Helmintos. Instituto de Biología, Universidad Nacional Autónoma de México, CONABIO, México. 211 pp.
- León-Règagnon, V.** 1992. Fauna helmintológica de algunos vertebrados acuáticos de la Ciénaga de Lerma, Estado de México. *Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoológica* 63:151–153.
- Miller, E. L.** 1930. Studies on *Glypthelmins quieta* Stafford. *Journal of Parasitology* 16:237–243.
- O'Grady, R. T.** 1987. Phylogenetic systematics and the evolutionary history of some intestinal flatworm parasites (Trematoda: Digenea: Plagiorchioidea) of anurans. Ph.D. Thesis, University of British Columbia, Vancouver, B.C., Canada. 210 pp.
- Prudhoe, S., and R. A. Bray.** 1982. *Platyhelminth parasites of the Amphibia*. Oxford University Press, Great Britain. 217 pp.
- Pulido, F. G.** 1994. Helmintos de *Rana dunni*, especie endémica del Lago de Patzcuaro, Michoacán, México. *Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoológica* 65:205–207.
- Stafford, J.** 1905. Trematodes from Canadian vertebrates. *Zoologischer Anzeiger* 28:681–694.
- Sullivan, J. J.** 1976. The trematode genus *Glypthelmins* Stafford, 1905 (Plagiorchioidea: Macroderoididae) with a redescription of *G. facioi* from Costa Rican frogs. *Proceedings of the Helminthological Society of Washington* 43:116–125.
- Yamaguti, S.** 1971. *Synopsis of Digenetic Trematodes of Vertebrates I*. Keigaku Publishing Co., Tokyo. Japan. 1,074 pp.